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Hydrogen production using an algae photoelectrochemical cell

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ABSTRACT

In this paper we present a photoelectrocatalytic-enzymatic hybrid system for simultaneous hydrogen production and organic pollutants' reduction. The destruction of the antibiotic Chloramphenicol, which acts as a model pollutant, will take place in the anodic compartment, while simultaneously hydrogen is produced in the cathode compartment of an algae photoelectrochemical cell. The photogenerated electrons in a TiO_2 anode are transferred to the cathode, where the hydrogenase enzymes derived from the algae *Chlamydomonas Reinhardtii* strain CC-124, catalyze the reduction of the H^+ species to H_2. Parameters like the growing medium, detergent, electron relay and algae concentration have been optimized. Fifty percent reduction in the organic carbon content and almost complete destruction of the Chloramphenicol molecule is possible at the anode under photoelectrocatalytic conditions. Simultaneously in the cathode compartment and in the presence of the algae culture *C. Reinhardtii* strain CC-124, 216 μM H_2 is produced over a reaction time of 450 min, using 0.4 mM Triton X 100 for the cell membranes rapture and 0.05 mM Methyl Viologen (MV^{+2}), which acts as an electron relay, in a Tris-acetate-phosphate (TAP) sulphur free medium. The expression of the hydrogenase enzymes under photoheterotrophic and sulphur deprived conditions has been confirmed by a reverse transcription-polymerase chain reaction (RT-PCR).

1. Introduction

Two of the most important problems that humanity faces during the last 50 years are the decrease of fossil fuels and the increase of the environmental pollution. The global energy consumption increases exponentially [1,2], while the domestic and agricultural/industrial activity, apart from the unreasonable energy and water consumption, has led to an increase in the amount of pollutants.

The exploitation of solar energy, as the largest renewable carbon free resource, represents an obvious idea for the solution of these problems, as it is abundant and exceeds by far the human needs, but must be collected and efficiently transformed into useful forms such as heat, electricity and fuels. Especially the production of a clean, efficient and storable "solar fuel" would be an important breakthrough.

Water splitting and hydrogen production represents a viable and environmentally friendly option as a future fuel supply, since it is non-polluting and its combustion generates again water [3,4]. Although hydrogen seems to be the perfect alternative fuel, there is no real environmental benefit right now, because most of it is extracted from fossil fuels. Therefore, many research activities focus on the development of more cost-effective and energy efficient methods, such as thermal/catalytic, photobiological, photochemical, electrochemical etc. [5–7], producing hydrogen from water or biomass, as a fuel and a source of electricity. The use of solar light for hydrogen production should be an ideal method for sustainable energy production using renewable solar energy [8,9].

Photosynthetic hydrogen generation from water has been known for 70 years and it is a property of many phototrophic organisms (green algae, cyanobacteria and photosynthetic bacteria) that can convert sunlight to useful, stored chemical energy [10,11]. More specifically, unicellular green algae exhibit the ability to act as biological catalysts in the production of hydrogen from water through the induction of enzymes known as hydrogenases, which can covert the released protons to hydrogen. In such in vitro biological systems the electrons needed for the hydrogen reduction are donated through complex electron transport systems such as Photosystem I (PSI), Photosystem II (PSII) and Ferredoxin (Fd) [12,13]. Theoretically, in an artificial photosynthetic system the replacement of these complex enzymatic systems can be done by a simple semiconductor electrode (e.g. TiO_2), which is able by the appropriate irradiation to produce electrons/holes pairs for enzymatic hydrogen production and organic pollutants oxidation respectively [14–16].

In addition to the energy problems humanity faces nowadays, water purification research has been extensively growing during the last decades, as water quality control and regulations

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against hazardous pollutants have become stricter in many countries. The so-called advanced oxidation technologies (AOTs) are among the most effective chemical oxidation processes [17,18], and are currently gaining significant importance in water treatment applications, as they can provide almost total inactivation of pollutants. Among these, the solar photocatalytic/photoelectrocatalytic oxidation using TiO_2 has shown great promise for pollution reduction [19,20], allowing the contribution of renewable sources of energy (solar energy) to the process of cleaning and restoring the environment. In this paper we present a photoelectrocatalytic/enzymatic hybrid system, which by the destruction of toxic and detrimental pollutants present in wastewaters produces simultaneously hydrogen. The principle of operation of this hybrid system is described elsewhere [21] and aims to replace the complex biological systems (PSI, PSII, Ferredoxin), with a simple semiconducting electrode. More specifically in the anodic compartment of a photoelectrochemical cell the oxidation of Chloramphenicol [22] (a broad spectrum antibiotic which acts as a model pollutant), will take place on an illuminated TiO_2 surface by the photogenerated holes. The photogenerated electrons, on the other hand, will then be transferred to the cathode, where the hydrogenase enzyme derived from the algae *C. Reinhardtii* strain CC-124, will catalyze in the dark and in the presence of Methyl Viologen (MV^{+2}) as redox mediator, the reduction of the H^+ species to H_2 [23]. This methodology should provide useful energy production with simultaneous pollution reduction, leading to an attractive and effective way of trapping energy from renewable resources in a sustainable way.

Some similar studies, concerning the hydrogen production by coupling photoelectrochemical/biological processes are present in the literature [16,24], but in most of these studies purified hydrogenase enzymes immobilized on the surface of a cathode were used. In the contrary, in our system instead of the purified enzyme, the cathodic compartment contains algae cells under anoxic and sulphur deprived conditions, in combination with a surfactant (Triton X 100) and an electron relay (MV^{+2}). The results presented in this study show an increase in H_2 production in comparison to our previously submitted work, as a result of the optimization of different parameters such as, growth medium, as well as algae, detergent and electron relay concentrations.

2. Experimental

2.1. Chemicals

Chloramphenicol ($C_{11}H_{12}O_5N_2Cl_2$, 2,2-dichloro-N-[2-hydroxy-1 (hydroxyl-methyl)-2(4-nitrophenyl)ethyl]acetamide) and Methyl Viologen dichloride hydrate 98% ($C_{12}H_{14}Cl_2N_2 \times H_2O$, 1,1′-dimethyl-4,4′-bipyridinium dichloride) were products of Sigma Chemie GmbH and they were used as received. Triton X-100 ($C_{14}H_{22}O(C_2H_4O)_n$ (n = 9–10), Polyethylene glycol tert-octylphenyl ether was a product of Fluka BioChemika and was used as received.

TiO_2 used throughout this work was from Degussa (TiO_2 P-25, anatase/rutile: 3.6/1, BET surface area 56 $m^2\,g^{-1}$, particle size 25–30 nm). H_2SO_4 and NaOH used to adjust the pH, as well as anhydrous Na_2SO_4 were from Merck. Ti plates (0.5 mm thick) were from Alfa Aesar (99.5%, metals basis). Doubly distilled water was used throughout the work. Aqueous stock solutions of Chloramphenicol were prepared every week, protected from light and stored at 4 °C.

2.2. Ti/TiO_2 anode preparation and characterization

Ti specimes of geometric surface area 0.5 cm^2 for the characterization of the electrodes or Ti cylindrical foils of geometric surface area 50 cm^2 (10 cm × 5 cm) for the bulk photoelectrocatalytic experiments, were cut from 0.5 mm thick Ti plates and were etched for a few seconds in a HF/HNO_3 3:1 mixture before washed thoroughly with doubly distilled water. The deposition of TiO_2 P-25 onto the Ti substrate took place from a 2 g L^{-1} TiO_2 suspension using the dip coating process. This process was repeated as required to produce TiO_2 films with a loading of 1 mg cm^{-2}, which corresponds to a 6.5 μm thickness. Finally the working electrodes were prepared by annealing the particulate TiO_2/Ti specimens in air at 500 °C for 1.5 h, in a Carbolite CWF 1100 oven, in order to improve the crystallinity and the adhesion of the prepared films. According to our previous studies [25], the best results concerning the photoelectrocatalytic efficiency, among specimens treated between 400 and 700 °C and for time intervals 1.5 until 10 h, were obtained with electrodes annealed at 500 °C for 1.5 h. For this reason all photoelectrocatalytic experiments, concerning the Chloramphenicol degradation, were carried out by using these electrodes.

2.3. Strain and culture growth

C. Reinhardtii CC-124 wild type mt- [137c] were purchased from the Chlamydomonas Resource Center of Minnesota's University and grown photoheterotrophically in flat plastic culture flasks containing Tris-acetate-phosphate (TAP) medium, pH 7 at 24 ± 2 °C [26]. The cultures, while growing were illuminated from one side with cool-fluorescence lamps (4× Osram Deluxe 18 W) providing an average incident light intensity of 80 μE m^{-2} s^{-1} Photosynthetically Active Radiation (PAR). The cultures were grown to the late logarithm phase (0.5–1 × 10^7 cells mL^{-1}), which then were harvested and washed 2 times with TAP sulphur free medium by centrifugation (3 min at 2500 × g) [11,12]. In the sulphur-free medium the sulphate anions were substituted with an equivalent amount of chloride ions. The cells were re-suspended in TAP sulphur deprived medium to the desired concentration ranging from 0.5 × 10^5 to 3 × 10^7 cells mL^{-1} and transferred to a sealed cylindrical glass bottle, which is the cathodic compartment of the algae photoelectrochemical cell. The average incident light intensity for the sulphur deprived cultures was increased to 180 μE m^{-2} s^{-1} PAR and anoxic conditions were achieved usually after 15 h from the cultures' sealing. All solutions for the algae growth were prepared in sterilized by autoclave and filter purified water.

A carbon rod (a cylinder of 40 cm^2 surface area, height = 7.5 cm; i.d. = 1.7 cm) which acts as the cathode electrode, was also added in the sulphur deprived culture at this point and it was present in the process of the culture's anoxia. Cell number was measured with Countess, an automated cell counter, a product of Invitrogen, Life Technologies Corporation.

2.4. Bulk experimental setup and procedures

The reactor used as the algae photoelectrochemical cell (APEC) is shown in Fig. 1. The anodic compartment (Fig. 1A), a 500 cm^3 cylindrical cell (i.d. = 7.8 cm; height = 14.8 cm), contained 300 mL of a 0.1 M Na_2SO_4 solution and 10 mg L^{-1} Chloramphenicol. An Osram Dulux 9 W/78 UVA lamp was placed in a cylindrical borosilicate glass sleeve and introduced in the middle of the anodic compartment. The light intensity on the TiO_2 electrode surface was measured as 3.9 mW cm^{-2} (Solar Light Co. PMA 2100), while the flux of the UV-A photons was found to be 1.2 × 10^{-4} Einstein min^{-1} by ferrioxalate actinometry [27]. The TiO_2 electrode of 50 cm^2 surface area was fitted between the cylindrical sleeve and the inner wall of the cell, while Ag/AgCl was used as reference electrode. The cathodic compartment (Fig. 1B) is a sealed 260 cm^3 cylindrical cell (50 mL headspace volume) and it was described in Section 2.3. When the hydrogenase enzyme was produced under heterotrophic sulphur deprived and anaerobic conditions the two compartments were put together and they were separated by a polysulfone porous membrane (Gelatin) impregnated with a Nafion

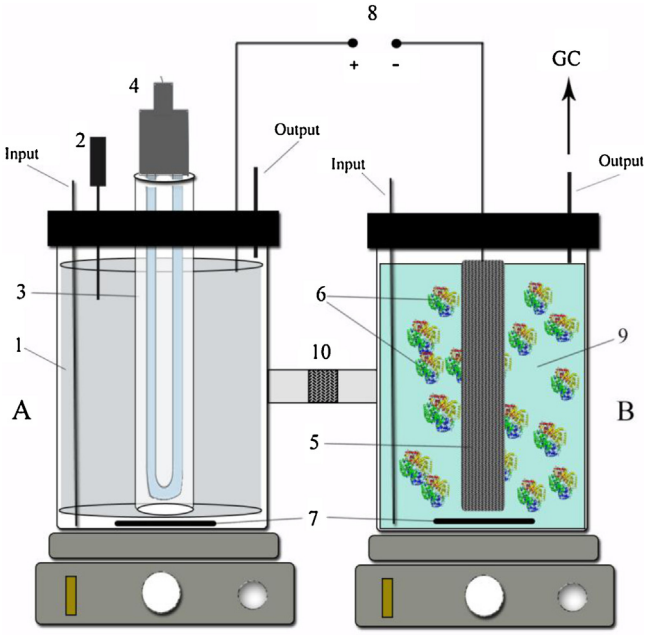

Fig. 1. Schematic representation of the algae photoelectrocatalytic reactor – (A) anode; (B) cathode; 1: working electrode (TiO_2/Ti cylinder); 2: Ag/AgCl reference electrode; 3: cylindrical sleeve where lamp (4) is inserted; 4: UV-A lamp; 5: counter electrode (Carbon rod); 6: hydrogenase enzyme; 7: magnetic stirrer; 8: potentiostat; 9: algae solution in Sueoka SF medium (contains MV^{+2} and Triton X-100); 10: electrolytic junction (Nafion membrane).

5% solution (Aldrich). The applied potential was obtained using the potentiostat Wenking POS 73 (Bank Elektronik). At specific time intervals samples from the anodic compartment were withdrawn in order to determine the concentration and the dissolved organic content (DOC) reduction of Chloramphenicol, while samples of the produced hydrogen were withdrawn directly from the cathodic compartment with a gas tight syringe and were measured with a gas chromatograph. All experiments were carried out at a temperature of 25 °C.

2.5. Analytical methods

Absorption spectra in the ultraviolet and visible range of the Chloramphenicol solution were recorded with a Shimadzu PharmaSpec UV-100 spectrophotometer, while a total organic carbon analyzer (Shimadzu Instruments, model V_{CSH} TOC Analyser) was used to monitor the dissolved organic carbon (DOC) reduction at the desired time intervals. The applied potential was obtained using the potentiostat Wenking POS 73 (Bank Elektronik), while for the differential capacity measurements the PGZ301 Voltalab 40 electrochemical system was used (Dynamic Electrochemical Laboratory).

Hydrogen and oxygen were analyzed by gas chromatography using a GC-Shimadzu 2014 chromatograph. The column used was a molecular sieve 5A (Supelco USA) and a thermal conductivity detector (TCD at 60 °C, oven at 35 °C). Helium used as the carrier gas.

The reproducibility of the optical absorption values was within ±5%, that of the dissolved organic carbon (DOC) ±10%, while that of the gas chromatography analysis ±5% for peak intensity and peak retention time.

2.6. Hydrogenase activity test

The hydrogenase activity assay was measured according to [11] for intact cells by the rate of hydrogen production from reduced MV^{+2} ($MV^{\bullet+}$) in 4 mL sealed glass vial. One unit of hydrogenase activity catalyzes the production of 1 μmol of H_2 min^{-1}.

2.7. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from 5×10^6 *Clamydomonas reinhardtii* CC-124 cells using TRIzol reagent (Life Technologies) according to the manufacturer's instructions. One microgram of total RNA was reverse transcribed using random primers (0.1 μg), 200 units M-MLV reverse transcriptase (Invitrogen), 40 units RNAse inhibitor, 5 mM $MgCl_2$ and 1 mM dNTP mix, in a total volume of 20 μL. The reaction tube was first incubated at 25 °C for 10 min, followed by incubation at 37 °C for 50 min. The reaction was inactivated by heating at 70 °C for 15 min.

To assess the expression of HYD1 ([Fe–Fe] hydrogenase), HYD2 ([Fe–Fe] hydrogenase), HYD3 ([Fe–Fe] hydrogenase/prelamin a binding protein), 1 μL of the reverse transcription reaction was used as template in subsequent PCR reactions in 50 μL reaction volumes. Parameters for polymerase chain reaction were as follows: denaturation at 95 °C for 40 s, annealing at 61 °C (HYD1 and HYD3) or 60 °C (HYD2) for 40 s, and extension at 72 °C for 50 s. PCR was performed with the addition of 8% dimethyl sulfoxide (DMSO). The sequence of the primers were as follows: for HYD1 (320 bp amplicon), sense primer: 5′-GATGTCGGCGCTCGTGCTGAAGCCCTG-3′, antisense 5′-CACCTCGTCAAAGCCGAGGCGGCGGAG-3′; for HYD2 (400 bp amplicon), sense primer: 5′-GATGGCGCTTGGTCTTCTTGCCGAGCTG-3′, antisense 5′-CAGCGTGTCAAACACCTGGTCGAAGCCG-3′; for HYD3 (330 bp amplicon), sense primer: 5′-GGCAATGGAGTTCTCCTCGACGATTCGCG-3′, antisense 5′-CGGGCTCAGACCAAAGTAGGCCGCCAG-3′; PCR products were electrophoresed on 1% agarose gels and visualized by staining with ethidium bromide.

3. Results and discussion

Before coupling both processes for the anodic Chloramphenicol photodegradation and the cathodic hydrogen production, a photoelectrochemical characterization of the prepared TiO_2 electrode, as well as an investigation of the cathode compartment containing the algae cells is essential, with the scope to find out the optimal working conditions of the hybrid system.

3.1. Electrochemical/photoelectrochemical behavior of the TiO_2/Ti anode

The electrochemical behavior of many semiconductor materials has been interpreted in terms of Schottky barrier behavior [28,29]. Two important parameters which influence their electronic and electrochemical properties are the flat band potential (V_{fb}) and the donor density (N_D). A simple way to calculate the values of these parameters is by measuring the differential space charge capacitance, C_{sc}, versus the interface potential, V and using the classical Mott–Schottky relationship, which describes the total capacitance of the semiconductor/electrolyte interface. In the case of an n-type semiconductor, such as TiO_2, this relationship is given according to Eq. (1)

$$\frac{1}{{C_{sc}}^2} = \frac{2}{\varepsilon\varepsilon_0 e N_D}\left(V - V_{fb} - \frac{kT}{e}\right) \quad (1)$$

where ε is the average value of the semiconductor dielectric constant (~75 for TiO_2 [30]), ε_0 ($F\,m^{-1}$) the permittivity of the free space charge, N_D (cm^{-3}) the donor density and e (C) the absolute value of the electron charge. V (volts) corresponds to the external applied potential, while T (K) and k ($J\,K^{-1}$) are the absolute temperature and the Boltzmann constant, respectively.

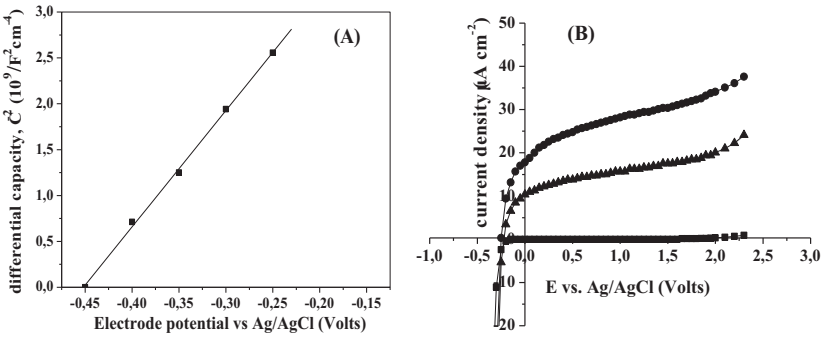

Fig. 2. (A) Mott-Schottky plot of the TiO_2 film in 0.1 M Na_2SO_4, at 500 Hz and pH = 5.6. (B) *I–V* voltammograms for the TiO_2 electrode, at pH = 5.6 in 0.1 M Na_2SO_4 in the dark (■) and under illumination (▲) and in 0.1 M Na_2SO_4 + 10 mg mL^{-1} Chloramphenicol under illumination (●).

Fig. 2A presents the Mott–Schottky plot for a TiO_2/0.1 M Na_2SO_4 (pH = 5.6) electrochemical junction in the dark at 500 Hz. It can be seen that, for an appreciably wide potential range, the C^{-2} vs. V plots show good linearity. From the intercept of this line with the potential axis and according to Eq. (1) a value of −0.410 V vs. Ag/AgCl or −0.19 V vs. the normal hydrogen electrode (NHE) at pH = 5.6, for the V_{fb} of the semiconductor electrode has been calculated, while the donor density, N_D, which corresponds to the frequency of 500 Hz, according Eq. (1) is 1.511×10^{18} cm^{-3}. These experimental values are in good agreement with values reported in the literature [31].

The current–potential curves of the TiO_2 electrode in 0.1 M Na_2SO_4 in the dark and under UV-A illumination, recorded within the potential range of −0.5 and +2.2 V vs. Ag/AgCl are given in Fig. 2B.

It can be seen that the TiO_2 electrode shows in the potential range of −0.2 to +2.0 V a nearly perfect blocking characteristic, typically for an n-type semiconductor, while an increase in dark current density for potentials higher than +2.0 V has been observed as a result of the water decomposition and oxygen evolution. Likewise, no current was observable in the presence of Chloramphenicol in the dark (not shown in the figure).

In the contrary, upon illumination a significant increase in the anodic current density above −0.2 V occurred. According to the literature [28,29], by illuminating a semiconductor-electrolyte interface with light energy greater than that of its band gap energy, electron–hole pairs (e^-/h^+) are generated in the space charge layer of the semiconductor. The simultaneous application of a bias potential positive to the flat-band potential produces a bending of the conduction and valence bands causing a more effective separation of the photogenerated carriers within the space charge layer and increases the photocurrent (I_{ph}) that begins to flow and likely promotes better the oxidative degradation process. The potential gradient efficiently forces the photogenerated electrons to arrive at the counter electrode and leave the photogenerated holes to react with H_2O/OH^- to give rise to $OH^\bullet$ radicals, which can further react with the organic molecules adsorbed onto the TiO_2 surface.

These oxidation reactions are accompanied by a reduction reaction, due to the photogenerated electrons transferred to the cathode compartment (e.g. reduction of water, Eq. (2))

Cathode (counter electrode):

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (2)$$

or as we will see, in our case by the enzymatic H^+ reduction from reduced MV^{+2}.

$$MV^{+2} + e^- \rightarrow MV^{\bullet+} \quad \text{(cathodicreaction)} \quad (3)$$

$$MV^{\bullet+} + H^+ \xrightarrow{\text{hydrogenase}} MV^{+2} + \frac{1}{2}H_2$$

$$\text{(homogenous enzymatic reaction)} \quad (4)$$

3.2. Hydrogenase enzyme expression at the cathode compartment

As described in Section 2.3 the sealed cylindrical cell where the algae cells grow under anaerobic and sulphur-deprived conditions is the cathode compartment of the hybrid system. Before coupling it with the anodic compartment, the hydrogen production under purely photobiological conditions was investigated in order to confirm the ability of *C. Reinhardtii* to express the hydrogenase enzyme and to produce hydrogen. In addition to the photobiological hydrogen production, RT-PCR was employed using cells' RNA so as to further confirm the formation of the hydrogenase enzymes, which act as catalyst in the cathodic hydrogen production.

Melis et al. reported that sulphur deprived algae cells can lead to sustained hydrogen photoproduction. A partial and reversible inhibition of photosystem II (PSII) is caused in sulphur-deprived culture under high intensity light, which has a small effect on cellular respiration, but the D1 protein of PSII, which is essential for photosynthesis, cannot be replaced and turns over due to continuous photodamage leading to a gradual loss of O_2 evolution. When the O_2 evolution rate is lower than the respiratory O_2 consumption rate, anaerobiosis is established [12]. Anaerobiosis is mandatory for hydrogenase to be inducted and the expression of 3 iron hydrogenases in algae cells has been reported HYD1, HYD2, HYD3 [32–34]. These enzymes in the absence of O_2 redirect the electron pathway from carbon fixation (PSI) in the chloroplast, to proton reduction resulting in hydrogen production for several days. The overall reactions involve the oxidizing site of PSII where the green algae can extract electrons from the water molecules and eventually transport these to the Fe-S protein Ferredoxin (Fd) on the reducing side of PSI. The reversible hydrogenase accepts the electrons from the reduced Ferredoxin and donates them to 2 H^+ to produce one H_2 molecule according to Eq. (5) [12].

$$2H^+ + 2Fd^- \overset{\text{hydrogenase}}{\longleftrightarrow} H_2 + 2Fd \quad (5)$$

Our experiments confirm such a behavior as shown in Fig. 3A and in contrast to purified hydrogenases used in the literature [14,16,24] we exploited the ability of *C. Reinhardtii* to express the enzymes in sulphur deprived algae cultures and utilize them in the cathodic enzymatic hydrogen production in the dark. By this, the complex step of enzyme induction and purification can be avoided.

It is also evident from Fig. 3A that the use of TAP medium for the algae growth, instead of Sueoka medium used in our previous work [21] leads to a significant increase in the hydrogen production, as well as, in the lag time for anaerobic conditions to be induced. As reported in [21], in Sueoka medium 13%, v/v H_2 was produced after 2 days and the lag time was 1 day, while in the present study at the same time the TAP medium leads to a 35%, v/v H_2 production and the lag time for anaerobiosis to establish was reduced to some hours after the sealing of the cultures. This enhancement is also

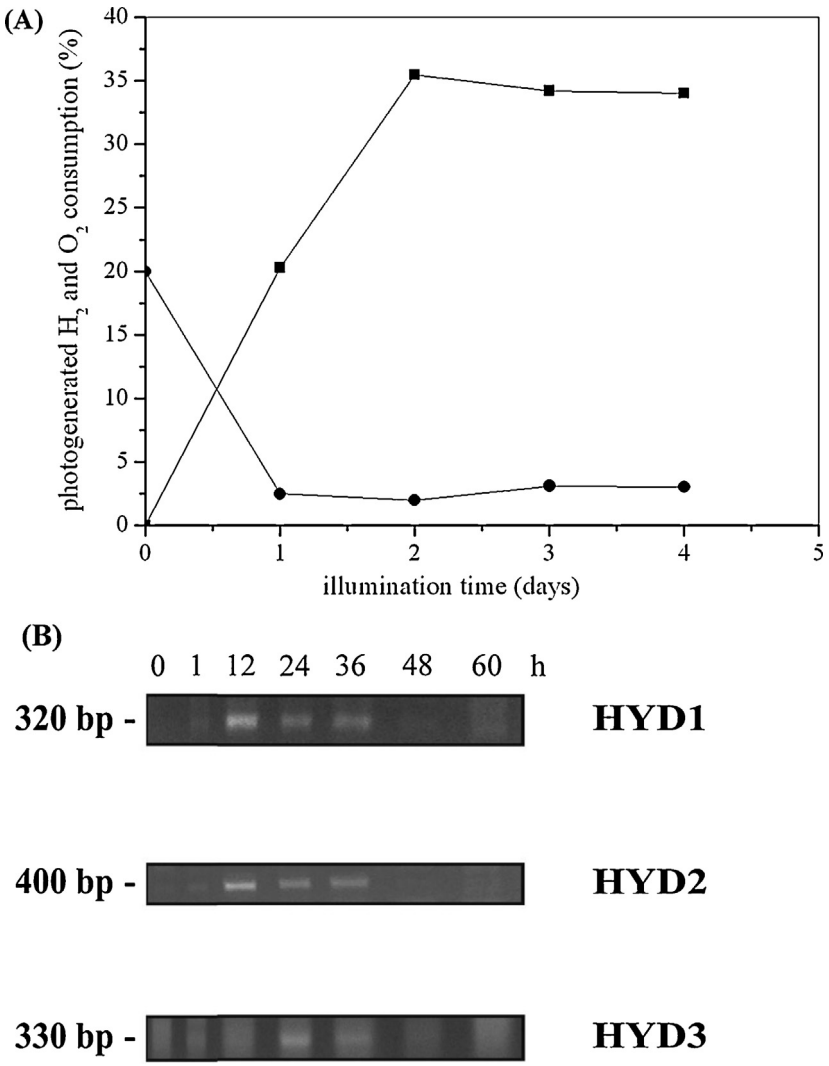

Fig. 3. (A) Hydrogen production (■) and oxygen (●) consumption in sulphur-deprived algae culture of 1×10^7 cells mL^{-1}, (B) electrophoresis showing the RT-PCR products from all amplifiable cDNAs.

related to the growth rate of the algae cultures, which under the same experimental conditions grew almost 3 times more in TAP than in the Sueoka medium.

In addition to the hydrogenase activity test, the enzyme's induction in anaerobic conditions was also confirmed by RT-PCR using total RNA from *C. reinhardtii* cells. Fig. 3B shows the expression of the 3 hydrogenases (HYD1, HYD2 and HYD3) whose specified role in hydrogen production is not fully clarified [34,35]. HYD1 and HYD2 are expressed 12–36 h following oxygen deprivation, with their expression reaching a maximum at 12 h, whereas HYD3 is expressed somehow later, with its expression reaching a maximum at 24 h following oxygen deprivation. These findings are in agreement with the hydrogen production timeframe and they show that the optimum time for the connection of the cathode with the anode is around 12 h after the sealing of the cathode. At that time, the hydrogenase enzymes are fully expressed in the cathodic compartment and can act as catalyst for the enzymatic hydrogen production.

3.3. Chloramphenicol degradation under simultaneous hydrogen production using the algae photoelectrochemical cell (APEC)

Once the hydrogenase enzyme was produced under anaerobic and sulphur deprived conditions, as it is described in Section 3.2, the cathodic compartment was placed in the dark and connected with the anodic one. Anode and cathode were separated by a Nafion impregnated membrane and an external bias of +0.6 V vs. Ag/AgCl was applied by the potentiostat to the algae photoelectrochemical cell (APEC).

Following this, we studied the ability of the Ti/TiO_2 photoanode to oxidize the Chloramphenicol molecule under simultaneous production of hydrogen at the cathode compartment. A more detailed description of its photocatalytic degradation can be found in our previous paper [22]. Here we use the selected antibiotic as a model pollutant and investigate its degradation using the photoelectrochemical oxidation.

The performance of the TiO_2 electrode, with respect to the degradation and mineralization of Chloramphenicol as a function of the illumination time is shown in Fig. 4. The decomposition of the Chloramphenicol molecule was monitored spectrophotometrically by its absorption peak intensity at 278 nm and by its organic content reduction, expressed as DOC. The relative decrease shown, is the ratio of Chloramphenicol or DOC concentration at time t to its initial one in the solution at $t = 0$. As indicated in Fig. 4, when applying an anodic potential of +0.6 V, the photodegradation of Chloramphenicol after 8 h of illumination of the TiO_2 electrode was complete, while at the same time the extent of mineralization expressed as the dissolved organic carbon (DOC) reduction leads to a ~60%

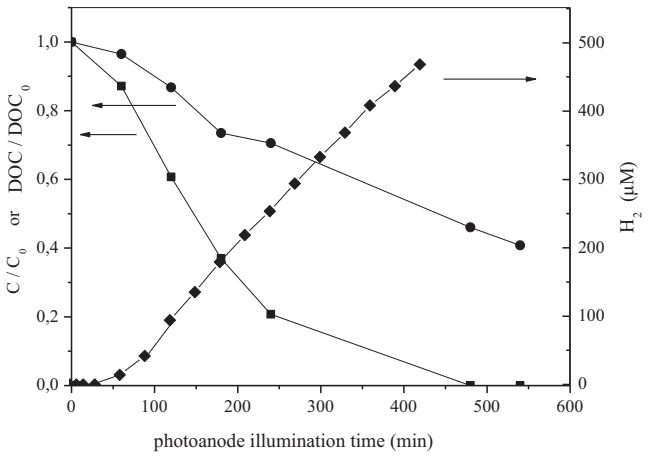

Fig. 4. Degradation (■) and mineralization (●) of Chloramphenicol on a TiO_2 electrode vs. illumination time at +0.6 V vs. Ag/AgCl in 0.1 M Na_2SO_4: the Chloramphenicol concentration was 10 mg L^{-1} (8.45 mg L^{-1} DOC). (♦) Hydrogen production in TAP medium, 3×10^7 cells mL^{-1}, in the presence of 3.2 mM Triton X 100 and 1 mM of MV^{+2}.

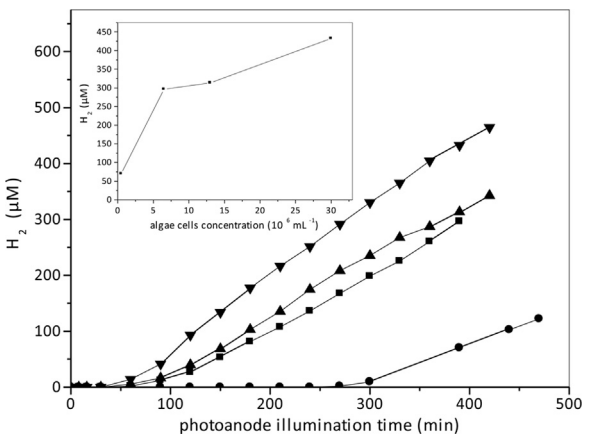

Fig. 5. Effect of the algae concentration on hydrogen production in the presence of 1.6 mM Triton X 100 and 0.5 mM MV^{+2}: 5×10^5 cells mL^{-1} (●), 6.5×10^6 cells mL^{-1} (■), 1.3×10^7 cells mL^{-1} (▲), 3×10^7 cells mL^{-1} (▼) (inset: hydrogen production as a function of the algae cells concentration in the algae photoelectrochemical cell after 390 min of anode illumination).

reduction of the initial carbon content of Chloramphenicol, showing the presence of difficult to degrade intermediates.

Fig. 4 shows also the simultaneous hydrogen production in the cathode compartment from the hydrogenase enzyme in the dark. As can be seen, 464 μM of hydrogen are produced at 420 min of illumination of the photoanode.

As already mentioned, the main purpose of the presented system for the photoelectrochemical/enzymatic hydrogen production, is to replace the natural electron transfer systems (PSI, PSII, Fd) with an artificial one, using the photoelectrochemical oxidation, and to provide the hydrogenase enzyme with photoelectrons derived from the reaction in the anode compartment. From Fig. 4 it is evident that the natural electron transfer systems can be successfully replaced by the TiO_2 anode. The illumination of the semiconductor electrode produces electrons which are being transferred to the cathode, where MV^{+2} according to Eqs. (3) and (4) and probably buffer ingredients are then able to provide the electrons to hydrogenase enzyme for hydrogen production [14,36].

In our previous study [21], we reported the production of 21.3 μM of H_2 in Sueoka medium under the same experimental conditions. The 20-fold enhancement in the present case is attributed to the change in the growth medium of the cells from Sueoka to TAP and indicates better buffer characteristics of the TAP medium.

The growth of the algae cells in the TAP medium under the same conditions was also 10 times higher and the hydrogenase enzyme shows a catalytic activity of more than 400 min in contrast to the Sueoka medium, where its activity was limited to 45 min. Additionally, in the case of the Sueoka medium the hydrogen production starts almost immediately, while in TAP medium there is a lag phase of about 90 min. This can probably be attributed to a slower rate in the surfactant's effect in the opening of the cell membranes. Overall the TAP medium results in a greater stability for the activity of the hydrogenase enzyme when compared to its Sueoka analog [21].

In an attempt to prolong the enzyme's activity and the optimization of the presented system for hydrogen production, the effect of the detergent and of the electron mediator in the cathode compartment is studied and presented below. In all cases, the anode conditions and performance are kept as presented above.

3.4. Effect of the algae concentration in the hydrogen production

In this section the effect of different initial algae concentrations on hydrogen production was investigated. It is expected that with increasing cells concentration more hydrogenase enzyme is produced leading to an increasing hydrogen production. In this set of experiments 1.6 mM Triton X 100 and 0.5 mM MV^{+2} were used, while the applied potential at the anode was +0.6 V vs. Ag/AgCl. The results are given in Fig. 5.

As expected, hydrogen production increases with increasing cell concentration. This is attributed to the higher hydrogenase activity present in the cathodic compartment, which is directly linked to the cells concentration. A linear relationship (not shown here) was found between the cells' concentration and the measured hydrogenase activity, which is in accordance with our previous results.

Another way to present this increase is by plotting the total amount of hydrogen produced versus the algae cells concentration (inset of Fig. 5). With 0.5×10^6 cells mL^{-1}, 70 μM of H_2 were produced in 390 min, while a 10-fold increase in the number of cells, increases the hydrogen production in the same reaction time by a factor of ~4.2. Higher increase in the cells concentration leads to ~6.2 times increase in the produced hydrogen (432.6 μM), as can be seen in the inset of Fig. 5.

3.5. Effect of Triton-X 100 concentration in the hydrogen production

During this study specific amounts of Triton-X 100 (0–4.8 mM) were added to the cathode compartment with the aim to open the cell membranes and release the hydrogenase enzyme to the bulk [37]. This is also part of the novelty of the presented system in comparison to reported systems in the literature where purified enzymes were immobilized onto metallic substrates. In Fig. 6 the effect of the detergent's concentration on the cathodic hydrogen production is presented under simultaneous illumination of the TiO_2 anode.

As can be seen in the inset of Fig. 6 an increase in H_2 production is observed by increasing the Triton-X 100 concentration until 0.4 mM, while above this value the hydrogen production is reduced, leading to the conclusion that the hydrogenase enzyme activity is hindered by the surfactant's concentration higher than this value. According to the literature, a hydrophobic shell around enzymes from detergents or lipids increases their enzymic activity, but after a critical concentration this activity is strongly inhibited [38]. We can see such an effect in our case as well and it is probably due to an undesired strong shielding of the enzyme's active catalytic center from the surfactant or the formation of Triton X 100 – MV^{+2} micelles [38]. Blank experiments without surfactant, as can be seen

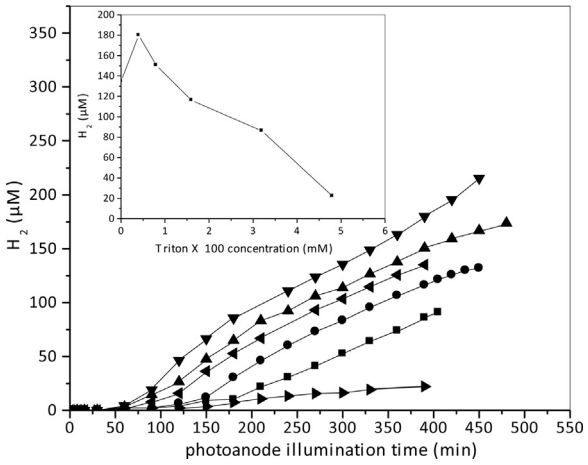

Fig. 6. Effect of the Triton-X 100 concentration on the hydrogen production in the algae photoelectrochemical cell, 0 mM (◄), 0.4 mM (▼), 0.8 mM (▲), 1.6 mM (●), 3.2 mM (■), 4.8 mM (►). In all cases ~1.5×10^7 algae cells mL^{-1} and 1 mM MV^{+2} were used (inset: hydrogen production after 390 min as a function of the detergent's concentration).

in the inset of Fig. 6, shows that hydrogen production is also possible, maybe as a result of the capability of the reduced MV^{+2} to penetrate the cell membrane [36].

3.6. Effect of MV^{+2} concentration in the hydrogen production

The role of MV^{+2} as an electron mediator in enzymic reactions is well known and widely discussed in the literature [38–40]. It has also been reported that viologen with shorter alkyl chains interacts electrostatically stronger with the negatively charged part of the proteins, producing so H_2 at a higher rate [41]. In our case MV^{+2} was also used as an electron relay between the carbon cathode and the enzyme with the scope to increase the efficiency of the charge transfer from the cathode to the hydrogenase in the solution. As it is mentioned in Section 1 and according to Eqs. (3) and (4) the reduction of MV^{+2} to $MV^{\bullet+}$ in our work comes from the photoelectrocatalytic oxidation of Chloramphenicol in the anode compartment and from the ability of the carbon electrode in the cathode to transfer the photogenerated electrons to MV^{+2}. No electron donors as dithionite were used. Fig. 7 shows the hydrogen production under different MV^{+2} concentrations versus the

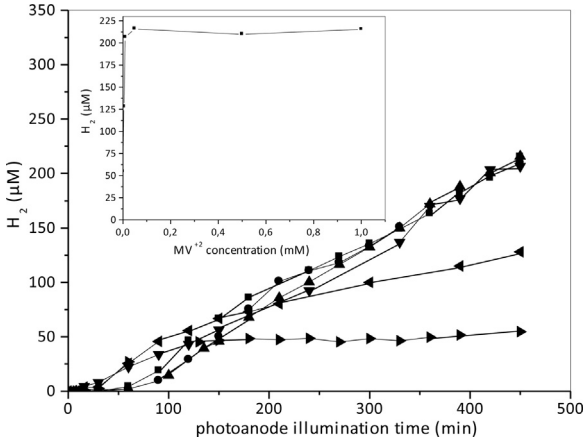

Fig. 7. Effect of the MV^{+2} concentration on the hydrogen production in the algae photoelectrochemical cell, 0 mM (►), 0.005 mM (◄), 0.01 mM (▼), 0.05 mM (▲), 0.5 mM (●), 1 mM (■).In all cases 0.4 mM Triton X 100 and ~1.95×10^7 cells mL^{-1} were used (inset: hydrogen production after 450 min as a function of the MV^{+2} concentration).

anode illumination time, while the inset shows the total hydrogen produced at the cathode compartment of the algae photoelectrochemical cell after 450 min of illumination of the TiO_2 anode at a +0.6 V applied potential under the same experimental conditions. It must be noted that blank experiments by MV^{+2} concentrations below 0.05 mM, showed also hydrogen production via pure photoelectrolysis (in the absence of algae cells). Therefore in the results presented in Fig. 7 the given enzymatic values are the difference between the total hydrogen and the pure photoelectrolytic one. As can be seen in all cases enzymatic hydrogen production was possible and the produced H_2 first increases sharply and then reaches a saturation value at higher concentrations of MV^{+2}.

It is of interest the fact that hydrogen is also produced in the absence of MV^{+2}. As seen in Fig. 7, 54.7 μM of H_2 were produced, but hydrogen evolution stops after the first 200 min of the anode illumination. This is probably due to electron transfer from reduced ingredients of the TAP medium to the chloroplast stroma of the algae cell, where the hydrogenase enzyme is believed to be active [35]. On the contrary, in the rest of the conditions hydrogen production continues for more than 400 min, because the reduced state of MV^{+2} ($MV^{\bullet+}$) was always present, so there is always the appropriate amount of the relay for the electron transfer from the MV^{+2} to the hydrogenase enzyme.

4. Conclusions

In this work we presented an algae photoelectrochemical cell, where simultaneously with the hydrogen production by algae cells in the cathode compartment, the photoelectrochemical oxidation of a model pollutant such as the antibiotic Chloramphenicol, took place. The scope of our study was to replace the very complex biological systems (PSII, PSI, Ferredoxin etc.) used by the natural photosynthetic processes for the production and transport of the electrons, with a semiconducting anode, where under illumination electron photogeneration will take place and the destruction of various pollutants will also be possible. Equally important is the idea to replace the costly platinum cathode used mainly for the electrocatalytic proton/water reduction to hydrogen, with the hydrogenase enzyme derived from the algae *C. Reinhardtii*. An additional goal was also the optimization and strengthening of our previously reported system. From the results in our present work we can concluded that 0.4 mM of Triton X 100 and 0.05 mM MV^{+2} are the optimum conditions for a 10-fold enhancement in the enzymic hydrogen production, in comparison to our previous work [21]. The enzyme's life and activity was also drastically prolonged with TAP sulphur free medium at a pH 7 in contrast to the Sueoka medium. At the same time the complete destruction and a 50% mineralization of the antibiotic Chloramphenicol was possible in the anode.

The combined system, which was tested under artificial illumination, effectively reduced the organic load and produced a useful energy carrier (H_2). This combination resulted in an innovative and effective operational system of hydrogen production and wastewater treatment. Additionally the use of solar light for the activation of the photoanode can offer economically reasonable and practical solutions to the processing of liquid waste under simultaneously hydrogen production. However further research is needed for the optimization of the proposed photoelectrochemical system, which can include the development of visible light sensitive anode materials, as well as hydrogenase enzymes that are less oxygen sensitive or even active under aerobic conditions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apcatb.2013.05.011.

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